

(19) World Intellectual Property Organization
International Bureau



(43) International Publication Date
8 September 2000 (08.09.2000)

PCT

(10) International Publication Number
WO 00/52146 A3

(51) International Patent Classification⁷: C12N 9/00, 9/14,
15/10, 15/62, 15/82, A01H 1/00, 5/00, G06F 17/50

(21) International Application Number: PCT/US00/05448

(22) International Filing Date: 3 March 2000 (03.03.2000)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
60/122,943 5 March 1999 (05.03.1999) US
60/142,299 2 July 1999 (02.07.1999) US
60/164,617 10 November 1999 (10.11.1999) US
60/164,618 10 November 1999 (10.11.1999) US

(71) Applicant (for all designated States except US): MAXY-
GEN, INC. [US/US]; 515 Galveston Drive, Redwood City,
CA 94063 (US).

(US). LASSNER, Michael [US/US]; 721 Falcon Avenue,
Davis, CA 95616 (US). YAMAMOTO, Takashi [JP/US];
42959 Nido Court, Fremont, CA 94539 (US). CARR,
Brian [US/US]; 3628 Thrush Terrace, Fremont, CA 94555
(US). NESS, Jon, E. [US/US]; 1220 N. Fair Oaks Avenue
#3115, Sunnyvale, CA 94089 (US). BERMUDEZ, Er-
icka, R. [ES/US]; Apartment 156, 1925 46th Avenue,
Capitola, CA 95010 (US).

(74) Agents: QUINE, Jonathan, Alan; The Law Offices of
Jonathan Alan Quine, P.O. Box 458, Alameda, CA 94501
et al. (US).

(81) Designated States (national): AE, AL, AM, AT, AU, AZ,
BA, BB, BG, BR, BY, CA, CH, CN, CR, CU, CZ, DE, DK,
DM, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL,
IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU,
LV, MA, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT,
RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA,
UG, US, UZ, VN, YU, ZA, ZW.

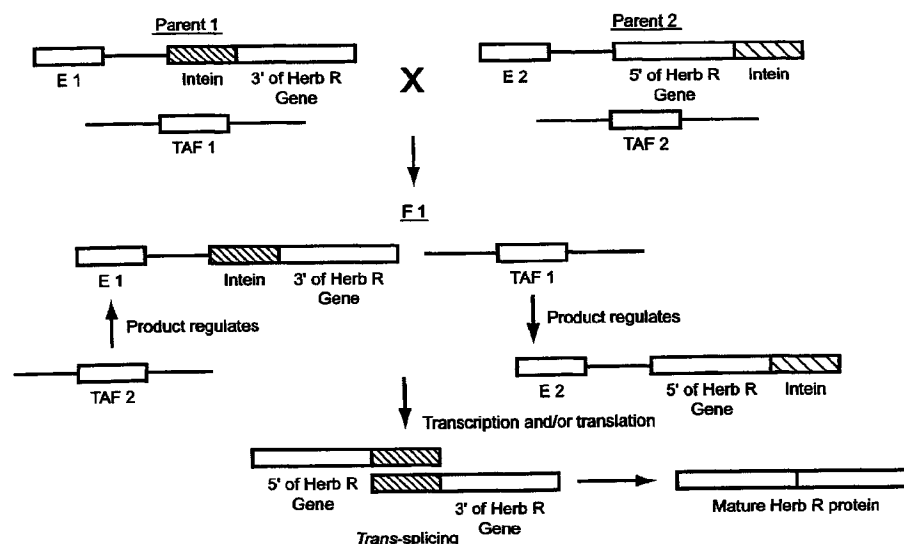
(72) Inventors; and

(75) Inventors/Applicants (for US only): PATTEN, Phillip,
A. [US/US]; 261 La Cuesta Drive, Menlo Park, CA 94028

(84) Designated States (regional): ARIPO patent (GH, GM,
KE, LS, MW, SD, SL, SZ, TZ, UG, ZW), Eurasian patent

[Continued on next page]

(54) Title: ENCRYPTION OF TRAITS USING SPLIT GENE SEQUENCES



(57) Abstract: Methods of unencrypting trait encrypted gene sequences to provide unencrypted RNAs or polypeptides. The invention also relates to methods of encrypting traits including splitting genes between two parental organisms or between a host organism and a vector. The gene sequences are unencrypted when the two parental organisms are mated or when the vector infects the host organism by *trans*-splicing either the split RNAs or split polypeptides upon expression of the split gene sequences. The invention also includes methods of providing multiple levels of trait encryption and reliable methods of producing hybrid organisms. Additional methods include those related to unencrypting engineered genetic elements to provide polypeptide functions and those directed at recombining non-overlapping gene sequences. The invention also includes integrated systems and various compositions related to the disclosed methods.



(AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).

(88) Date of publication of the international search report:
19 July 2001

Published:

— with international search report

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

INTERNATIONAL SEARCH REPORT

International Application No

PCT/US 00/05448

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WU H ET AL: "Protein trans-splicing by a split intein encoded in a split DnaE gene of <i>Synechocystis</i> sp. PCC6803" PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF USA., vol. 95, August 1998 (1998-08), pages 9226-9231, XP002137477 WASHINGTON., US ISSN: 0027-8424 page 9228, last paragraph -page 9229	1,3,5-7, 11,54, 57,58, 63-65
A	---	16,17, 32,33
X	SOUTHWORTH M W ET AL: "Control of protein splicing by intein fragment reassembly" EMBO JOURNAL, OXFORD, GB, vol. 17, no. 4, 1998, pages 918-926, XP002121550 ISSN: 0261-4189 page 919 page 923, column 2 -page 924	1,3,5,6, 10,12
A	---	13,46
X	SHINGLEDECKER K ET AL: "Molecular dissection of the <i>Mycobacterium tuberculosis</i> RecA intein: design of a minimal intein and of a trans-splicing system involving two intein fragments" GENE, BARKING, GB, vol. 207, no. 2, 30 January 1998 (1998-01-30), pages 187-195, XP004108918 ISSN: 0378-1119 page 192, column 2 -page 193 page 195	1,3
A	---	11
A	WO 92 13089 A (GEN HOSPITAL CORP) 6 August 1992 (1992-08-06) page 11, line 24 -page 12, line 2 page 28, line 32 -page 29, line 7 page 30, line 5 -page 31, line 20 figure 7	21,22, 24-30, 41-52, 108-110
A	---	46
	EVANS T C ET AL.: "Semisynthesis of cytotoxic proteins using a modified splicing element" PROTEIN SCIENCE, vol. 7, 1998, pages 2256-2264, XP002925638 US abstract	

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INTERNATIONAL SEARCH REPORT

International Application No
PCT/US 00/05448

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
E	<p>WO 00 17342 A (UNIV BOSTON) 30 March 2000 (2000-03-30)</p> <p>abstract</p> <p>-----</p>	<p>1,2,4-6, 11,12, 106</p>

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 00/05448

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☒ Claims Nos.: 118-119
because they relate to subject matter not required to be searched by this Authority, namely:

The subject-matter of claims 118-119 was considered to represent a mere presentation of information in the sense of Rule 39.1(v) PCT, and a computer program in the sense of Rule 39.1(vi) PCT, and therefore no search has been carried out for those claims.
2. ☐ Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:
3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

1-66, 106-110 completely and 118-121 partially

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest.
- ☐ No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

1. Claims: 1-66, 106-110 completely and 118-121 partially

A method of unencrypting trait encrypted gene sequences to provide at least one unencrypted RNA or polypeptide comprising transcribing a plurality of split gene sequences to provide a plurality of RNA segments, trans-splicing at least two RNA segments together to provide an unencrypted RNA or translating the plurality of RNA segments to provide polypeptide segments and trans-splicing at least two polypeptide segments together to provide an at least one first unencrypted polypeptide; parental organisms, host organism, vectors, split gene sequence libraries and an integrated system comprising a computer for use in said method.

2. Claims: 66-105 completely and 118-121 partially

A method for unencrypting engineered genetic elements to provide at least one unencrypted polypeptide function comprising post translational modification of said encrypted polypeptide; genetic elements, parental organisms, host organism, vectors and an integrated system comprising a computer for use in said method and polypeptides and organisms made by said method.

3. Claims: 111-117 completely,
118-121 partially and 122-123 completely

A method of recombining non-overlapping gene sequences comprising combining a plurality of gap nucleic acid sequences, which overlap two or more of the non-overlapping sequences, with a plurality of non-overlapping sequences; a composition comprising libraries of gap nucleic acids and an integrated system comprising a computer for use in said method.

INTERNATIONAL SEARCH REPORT

information on patent family members

International Application No

PCT/US 00/05448

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9507351 A	16-03-1995	US 5498531 A	12-03-1996
		US 5780272 A	14-07-1998
		US 6150141 A	21-11-2000
-----	-----	-----	-----
WO 9213089 A	06-08-1992	AU 653787 B	13-10-1994
		AU 1249992 A	27-08-1992
		CA 2100579 A	18-07-1992
		CN 1065681 A	28-10-1992
		EP 0567581 A	03-11-1993
		HU 72183 A	28-03-1996
		IE 920125 A	29-07-1992
		JP 6507308 T	25-08-1994
		KR 234840 B	15-12-1999
		NZ 241311 A	28-03-1995
		PT 100018 A	26-02-1993
		US 5641673 A	24-06-1997
		US 6071730 A	06-06-2000
		US 5882907 A	16-03-1999
		US 5863774 A	26-01-1999
		US 5866384 A	02-02-1999
		US 5849548 A	15-12-1998
		ZA 9200349 A	28-10-1992
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WO 0017342 A	30-03-2000	AU 6258399 A	10-04-2000
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